

Synthesis and Characterization of Hydroxyapatite Bioceramic

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Abstract

The overall objective of this research is to develop and characterize the hydroxyapatite (HA) bioceramics. In order to achieve these objectives, several aims have been developed to keep track the research in line. The projects aims are to synthesis hydroxyapatite (HA) at various temperatures (room temperature and boiling temperature) and to investigate the effect of temperature on hydroxyapatite (HA) powder such as purity, crystalline structure, reacting temperature and morphological characteristic (grain size, grain shape and diffusion between grains). Industrial grade crystalline HA powder has been successfully synthesis using the wet chemical precipitation method. Scanning electron microscope had showed that the synthesis Hydroxyapatite powder consists of fine grain, homogenous and uniform distribution under the influence of reflux temperature of 30°C and stirring rate of 350 rpm. The crystallites size for HA powders increased from 2.1868nm to 2.3422nm with increasing of the temperature from 800°C to 1200°C. It can be concluded that the wet precipitation route for sample S1.3c (reflux temperature: 30°C, Stirring rate: 350rpm, Sintering temperature: 1200°C) is the most suitable to synthesis HA powder over all others parameters.

Keywords: Hydroxyapatite, Bioceramic, Biomedical, Tissue Engineering

1.0 Introduction

Hydroxyl apatite or also called hydroxyapatite (HA) is a form of calcium apatite with the formula $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ naturally occurred in mineral form. Pure HA powder is white, while naturally occurred apatites can also brown, yellow or green coloration. HA has being used as a bioceramic material in bone grafting, bone tissue engineering and drug delivery system [Widyastuti, 2009]. HA is one of the most versatile bioceramic due to its excellent bioactive, biocompatibility to nature and ease bone bonding to the surrounding tissues. The HA implant also beneficial as it is acceptance by the body's immune system [M.Dasgupta Adak, 2004].

Recently, the role of HA in biomedical application is well known. Furthermore, the function of HA in this biomedical application is largely determined by its similarity in chemical structure with biological apatite, which comprises the mineral phases of calcified tissues in the enamel, dentine and bone [Nather A 2012].

Tissue engineering is often defined as application of engineering and medical science for design, synthesis, modifying, growth and regenerate living tissues. In the past decades, a number of implant materials based on calcium

hydroxyapatite, calcium phosphate, ceramics of these phosphates, bio-glasses and composites have been applied. These implants materials have been used in orthopedics, neurosurgery and dentistry [F. Habib, S. Alam, 2012]. The implant materials can exhibit biological affinity and activity to surround the host tissues when implanted [Sargolzaei Aval et al. 2006].

HA structure is very similar to bone apatite and hard tissue in mammals [Sharif Hussein Sharif Zein et al. 2012]. Bone apatite is the major component of inorganic phase of bone which play an important role in the calcination and reabsorption process of bone. Furthermore, calcium phosphate contain in the HA structure are widely studied and applied in medicine and oral biology due to the apatite-like structure of enamel, dentin and bones, called hard tissues [Shigeki Hontsu et al. 2012].

In recent years, ceramics are used to replace various part of the body especially bone and teeth which are classified as bioceramics [Widyastuti, 2009]. Polymer/ceramic composite materials based scaffold are being introduced into tissue engineering with the aims of improving mechanical strength and cell attachment (Nemati Hayati et al., 2011). HA is a attractive ceramic biomaterial with a composition and structure closes to natural bone mineral. Nevertheless, HA has showed good biocompatibility, osteoconductivity and osteoinductivity. There are increasing interests in the fabrication and investigation of porous PCL/nano-HA composite as scaffolds for bone TE. Moreover, composite scaffolds have shown promise by premineralizing the matrix, also resulting in improved osteogenesis. Therefore, the composite scaffold has become, and will continue to be an active player in the tissue regeneration process instead of simply a cell carrier or tissue template. More effort needed to be inserted to translate these next generation scaffolds to the clinical level.

The powders, porous blocks or beads of HA are used to fill the bone defects or voids. The bone filler provided a scaffold and enhance the rapid filling of the void by naturally forming bone and bone grafts. It also reduces the healing time and become parts of the natural bone structure [Qiu D et al. 2011].

2.0 Materials and Methods

2.1 Preparation of 1M $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ stock solution

Calcium hydroxide powder (37g) was dissolved in 500ml of distilled water at room temperature (30°C) or boiling temperature (220°C). The mixture of Calcium hydroxide

(1M) was then stirred for few minutes until the $\text{Ca}(\text{OH})_2$ completely dissolve and form free moving Ca^{2+} ion in the solution.

2.2 Preparation of 0.25M of $(\text{NH}_4)_2\text{HPO}_4$ stock solution

85wt% Ortho-phosphoric acid was added into a 1000ml of distilled water. The solution then stirred well so that the acid will dissolved with distilled water and form HPO_4^{2-} ion solution.

2.3 Chemical precipitation

The wet chemical precipitation route used to synthesis HA bioceramic powders are described in the process flowcharts **Fig. 1**. The $(\text{NH}_4)_2\text{HPO}_4$ was then burette drop by drop into the calcium hydroxide (CaOH) solution until the pH of the mixture turns 12.3 under the continuous stirring rate of 350rpm and 1100rpm.

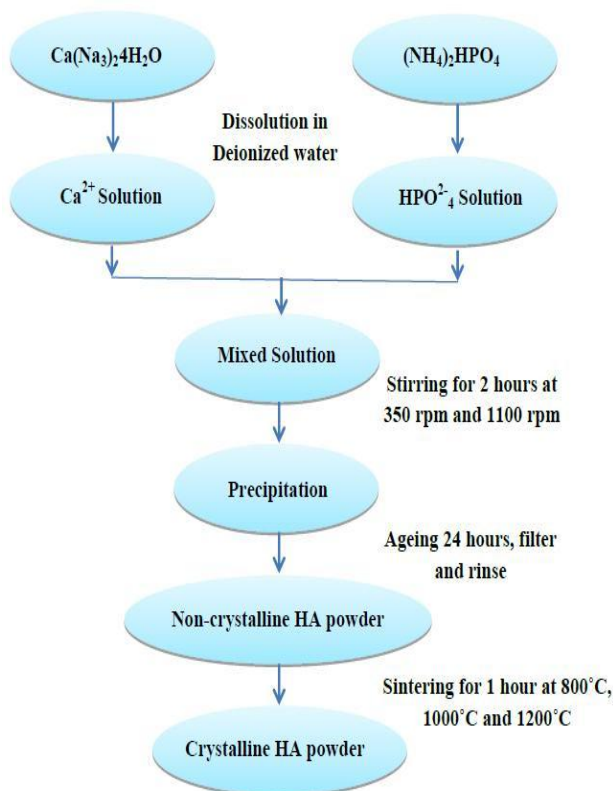


Fig. 1: Flow chart on preparation of the Hydroxyapatite using wet chemical precipitation method

About 150ml of 25% ammonia solution is being added in the mixture until the pH drop to 11.8 and this continue by added up 10g of Sodium Dodecyl Sulphate (SDS) powder to change the pH of the solution into pH of 11.0. The solution is continuously stirred for about 2 hours to mix the calcium and apatite structure.

The HA bioceramic is then left ageing for about 24 hours to left the product deposit slowly and form non-crystalline HA. The precipitate will be filter out and rinse with distilled water to remove any polluting agents before dry out in oven at 70°C for about 2 days.

Solid non-crystalline HA is then grinded using mortar and pestle to form fine and smooth HA powder, store in bottle and

labeled accordingly. The process is repeated again but this time SDS powder is not being added into the reacting mixture.

2.4 Sintering process

There are total of 8 samples prepared with different parameters ready to be sintered, see **Table 1**. The sintering process takes about 1 hour at different range of control temperature of 800°C, 1000°C and 1200°C in programmable furnace (Omron). Later, the samples were cold at the rate of 10°C/min for about 30 minutes.

No	Sample	Description		
		Present SDS	Reflux Temperature (°C)	Stirring rate (rpm)
1	S1.3	Yes	30	350
2	S4.1	Yes	30	1100
3	S7.1	Yes	220	350
4	S14.1	Yes	220	1100
5	S13.2	No	30	350
6	S16.2	No	30	1100
7	S19.2	No	220	350
8	S22.1	No	220	1100

The final products of 24 samples in **Table 2** were then prepared to characterize its purity and crystalline properties. Table 2: The final hydroxyapatite samples prepared at different parameters

No	Sample	Description			
		Present SDS	Reflux Temperature (°C)	Stirring rate (rpm)	Sintering Temperature (°C)
1	S1.3a	Yes	30	350	800
2	S1.3b	Yes	30	350	1000
3	S1.3c	Yes	30	350	1200
4	S4.1a	Yes	30	1100	800
5	S4.1b	Yes	30	1100	1000
6	S4.1c	Yes	30	1100	1200
7	S7.1a	Yes	220	350	800
8	S7.1b	Yes	220	350	1000
9	S7.1c	Yes	220	350	1200
10	S14.1a	Yes	220	1100	800
11	S14.1b	Yes	220	1100	1000
12	S14.1c	Yes	220	1100	1200
13	S13.1a	No	30	350	800
14	S13.1b	No	30	350	1000
15	S13.1c	No	30	350	1200
16	S16.1a	No	30	1100	800
17	S16.1b	No	30	1100	1000
18	S16.1c	No	30	1100	1200
19	S19.1a	No	220	350	800
20	S19.1b	No	220	350	1000
21	S19.1c	No	220	350	1200
22	S22.1a	No	220	1100	800
23	S22.1b	No	220	1100	1000
24	S22.1c	No	220	1100	1200

2.5 Characterization of Hydroxyapatite (HA) bioceramic powders.

The morphological characteristics of the HA powders were observed by Scanning Electron Microscopy (SEM) modelled FEI, 400K at 20kV of voltage and using low X-ray radiation.

The weight loss and reacting temperature of Hydroxyapatite powders were studied using Differential Scanning Calorimetry (DSC) & Thermo-Gravimetric Analysis (TGA) [M.Dasgupta Adak, 2011]. The composition, degree of crystallinity and size of crystallites of the Hydroxyapatite samples were studied with X-ray Diffraction.

3.0 Results and Discussions

Wet chemical precipitation route was chosen to synthesis the HA powders, as it is the most popular method and economical due to its low production cost [Dan Nicolae Ungureanu et al. 2011]. Furthermore, this process is only using distilled water as its main reacting solution it's involve no foreign elements and also produces ceramic with high homogeneity.

3.1 Visual Inspections

Prepared HA samples undergo the sintered process for about 2 hours at different control temperature, see Table 3. After being cool at 10°C/min, the samples are be inspecting its appearance. Table 3: Visual inspection results of various samples.

No	Sample	Sintering Temperature (°C)	Observation
1	S1.3a	800	White powder with rough texture
2	S1.3b	1000	Light green powder with rough texture
3	S1.3c	1200	Greenish powder with rough texture
4	S4.1a	800	White powder with rough texture
5	S4.1b	1000	Light green powder with rough texture
6	S4.1c	1200	Greenish powder with rough texture
7	S7.1a	800	White powder with rough texture
8	S7.1b	1000	Light green powder with rough texture
9	S7.1c	1200	Greenish powder with rough texture
10	S14.1a	800	White powder with rough texture
11	S14.1b	1000	Light green powder with rough texture
12	S14.1c	1200	Greenish powder with rough texture
13	S13.1a	800	White powder with rough texture
14	S13.1b	1000	Light green powder with rough texture
15	S13.1c	1200	Light green powder with rough texture
16	S16.1a	800	White powder with rough texture
17	S16.1b	1000	White powder with rough texture
18	S16.1c	1200	White powder with rough texture
19	S19.1a	800	White powder with rough texture
20	S19.1b	1000	White powder with rough texture
21	S19.1c	1200	Light green powder with rough texture
22	S22.1a	800	White powder with rough texture
23	S22.1b	1000	White powder with rough texture
24	S22.1c	1200	White powder with rough texture

Fig. 2 shows the sample S14.1b before and after sintered at 1000°C. The HA samples turn from white powder to greenish colour which show almost the same colour as the manufactured HA powder from industry. The texture of the HA powder also change from smooth to rough and non-uniform in particle size.

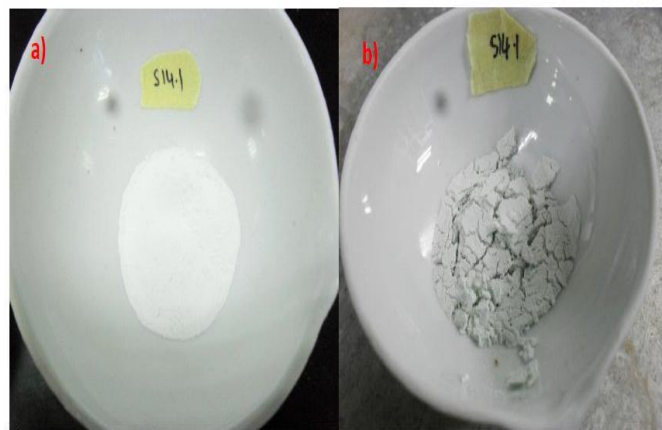


Fig. 2: Sample S14.1b a) before and b) after sintered at 1000°C. The colour of HA powder change from white to greenish colour.

Every sample that is sintered at 1000°C and 1200°C showed the same behavior after being sintered. Some samples have light green colour while others form a bit greenish powders. The different of green colour intensity is cause by the different reflux temperatures and the stirring rate of the mixture. Samples that are stirred at rate of 350 rpm and reflux at room temperature (30°C) had formed greenish powder, while samples stirred at rate of 1000 rpm in boiling solution (220°C) will give light green powder. Other than that, samples that are having SDS powder gives these results, see **Table 3**.

It can be concluding that SDS power had reacted at temperature above 1000°C to form pure crystalline HA powder. The 24 HA samples will be short listed before proceed to next characterization process. There are total 7 of new HA samples to be tested on SEM analysis and verified their morphological characteristic as shown in **Table 4** below.

Table 4: New HA samples to be tested on SEM analysis.

No	Sample	Observation
1	S1.3c	Greenish powder with rough texture
2	S4.1c	Greenish powder with rough texture
3	S7.1c	Greenish powder with rough texture
4	S13.1c	Light green powder with rough texture
5	S14.1b	Light green powder with rough texture
6	S14.1c	Greenish powder with rough texture
7	S19.1c	Greenish powder with rough texture

3.2 SEM Analysis

Fig. 3 shows the microstructure of sample S1.3c which having SDS powder and sintered at 1200°C. **Fig. 3(a)** consists of the particles with fine grain, homogenous and uniform distribution of particles which is same reported in [Dan Nicolae Ungureanu et al. 2011]. On the other hand, **Fig. 3(b)** under high resolution of 40 000 magnification, clearly

showed the grain boundaries between particles formed in present of SDS powder upon run the precipitation process. The average particles diameter of the grains is 304.8 nm. SDS powder becomes the reacting agent and helps to produce uniform HA apatite structure.

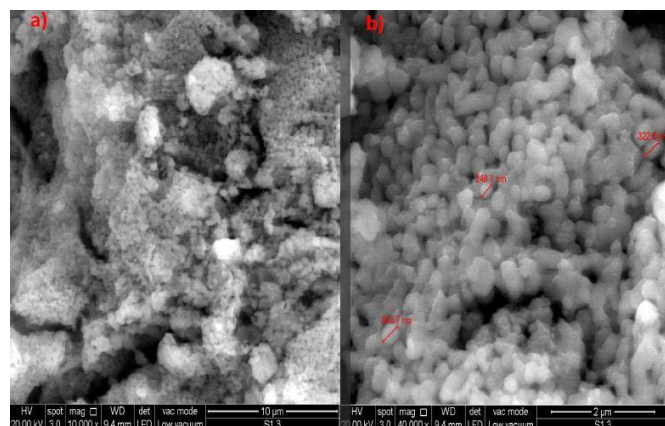


Fig. 3: The SEM images of **S1.3c**, **a)** magnification of 10 000x, **b)** magnification of 40 000x with the grain size measurement.

The morphological structure of the HA powder studied from the SEM images of sample **S19.1c** (no present of SDS powder and sintered at 1200 °C) shown in **Fig. 4** above. **Fig. 4(a)** consists of the particles with fine grain, homogenous and rough apatite structures. Existing of SDS powder had made the apatite structure accumulate randomly. The aggregates apatite, granular and seem diffuse each other in **Fig. 4(b)** showed different shapes as short, long columns and thick like plates under high resolution of 20 000 magnification. The average particle diameter of this microstructure is 403.7 nm which is usually recorded in many research.

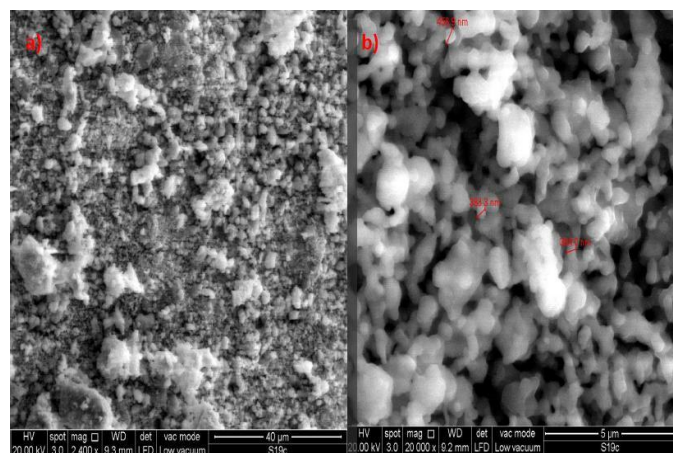


Fig. 4: The SEM images of **S19.1c**, **a)** magnification of 2 400x, **b)** magnification of 20 000x with the grain size measurement.

The average diameter size of the HA particles were recorded in **Table 5**. Manufactured HA (MHA) from the industry is also observed using the SEM analysis to be compared with the research findings, **Fig. 5**.

Table 5: The average diameter size of HA particles observed by SEM.

Sample	Average diameter of the HA particles (nm)
S1.3c	304.8
S4.1c	273.3
S7.1c	578.9
S13.1c	665.6
S14.1b	497.4
S14.1c	692.1
S19.1c	403.7
MHA	4 624.5

Unfortunately, the manufactured HA shows huge contrast of what the research aiming for, compare to the findings reported in many literatures.

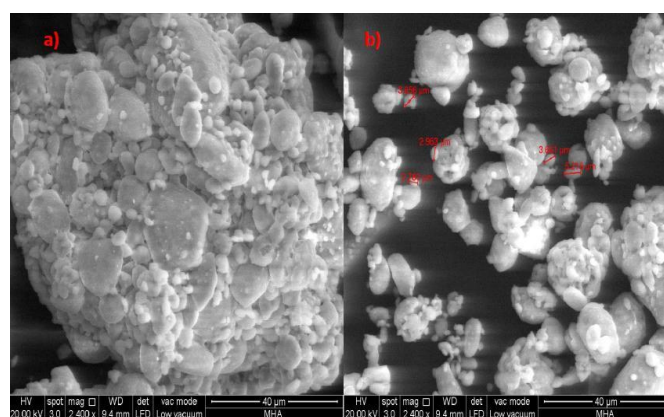


Fig. 5: The SEM images of **MHA**, **a)** magnification of 2 400x, **b)** magnification of 2400x with the grain size measurement.

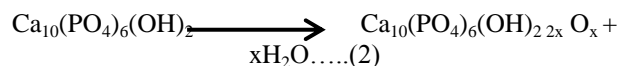
It has smaller diameter of grain size and the structure of the grains is smoother than the HA prepared in this research.

3.3 DSC & TGA analysis

Differential Scanning Calorimetry (DSC) & Thermo-Gravimetric Analysis (TGA) was used to study the thermal properties of the HA samples. The DSC analysis will estimate the reacting temperature of the HA samples while the TGA will measure the weight loss of the HA bioceramic. of sample S1.3c showed the weight loss of 98.64% at temperature between 350 °C to 750 °C which is happen due to the physical absorption of water [M.Dasgupta Adak, 2011]. The reacting temperature of the S19.1a sample is about 367.18°C. Sample S19.1a showed the weight loss of 92.75% at temperature between 300 °C to 500 °C which is happen due to the physical absorption of water [M.Dasgupta Adak, 2004]. The weight loss is due to the decomposition of MgCO₃ combined with the combustion of hydrocarbons over the range of temperature from 250 °C to 400 °C. The weight losses along the endothermic peak at 750 °C to 850 °C indicate the decomposition of CaCO₃ at this reaction.



Therefore, it is confirmed that the thermal analysis of HA basically contains CaCO_3 and small amount of MgCO_3 . Increasing temperature from 350 °C to 750 °C is resulted from the gradual dehydration of HA powder [M. Dasgupta Adak, 2011]. This can be explaining in the following reaction;



3.4 XRD analysis

The structural characterizations of the samples were carried out by X-ray Diffraction (XRD) with graphite nanochromatized $\text{Cu}_{K\alpha}$ radiation flux. All collected data were recorded in 2θ range of 20° to 60° with a step size of 0.02° 2θ . The X-ray analysis was used to analyses the present phases, the degree of crystallinity of Hydroxyapatite and its size of crystallites.

The crystallites size of HA powder are recorded in **Table 6** obtained from the crystallinity phase fraction equation. The crystallinity degree increases from 91.0922% to 96.5299% with increasing of heat treatment temperatures 1200°C. The degree of crystallinity of MHA is recorded as 91.0592% almost similar to sample S1.3c. It can be conclude that the wet precipitation route for sample S1.3c is the most suitable to synthesis HA powder over all the parameters.

The crystallite size, τ , of HA powder has been calculated based on Scherer's equation

$$\tau = (K \cdot \alpha) / W \cdot \cos(\theta), \quad [\text{nm}] \quad \dots (3)$$

Where:

K = constant dependent on crystallite shape, FWHM of spherical crystals with cubic symmetry;

λ is the wavelength of monochromatic radiation

W is defined as the full width of peak from the intensity distribution pattern measured at half of the maximum intensity value. This value is the difference between two 2θ values, in radians;

θ is the Bragg angle, in degree [D. Nicolae U., N. Angelescu, n.d].

Table 7 shows the Crystallite size, τ , of the synthesis Hydroxyapatite powder. When the temperature increased, the hydroxyapatite peaks became sharper, due to crystal growth [D. Nicolae U., N. Angelescu, n.d]. The crystallites size for Hydroxyapatite powders, according to Scherer's formula shows a proportional increase of crystallite size from 2.1868 nm to 2.3422 nm with increasing of the temperature from 800°C to 1200°C.

4.0 Conclusions

Crystalline HA powder has been successfully synthesis using the wet chemical precipitation method. This method produce HA powder naturally with light greenish colour similar to the manufactured HA powder from industry. Scanning electron microscope had proved that the synthesis Hydroxyapatite powder consists of fine grain, homogenous and uniform distribution under the influence of reflux temperature of 30°C and stirring rate of 350 rpm. The HA powder also react at

temperature above 367.18°C and obtained high percentage of weight loss of 98.78°C at endothermic range of 250°C to 400°C. The major phase is hydroxyapatite, which is confirmed by comparing data obtained with the ICDD - PDF2. The crystallinity degree increases from 91.0922% to 96.5299% with increasing of heat treatment temperatures 1200°C. The crystallites size for Hydroxyapatite powders, according to Scherer's formula shows a proportional increase of crystallite size from 2.1868 nm to 2.3422nm with increasing of the temperature from 800°C to 1200°C. Again it can be conclude that the wet precipitation route for sample S1.3c is the most suitable to synthesis HA powder over all the parameters.

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